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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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MAY 15 2001

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

To: Mike Mendelsohn, Regulatory Action Leader
Biopesticides and Pollution Prevention Division, 7511C

From: Robyn Rose, Entomologist Robyn Rose 5/15/01
Biopesticides and Pollution Prevention Division, 7511C

Peer Review: Zigfridas Vaituzis, Ph.D., Microbiologist Zigfridas Vaituzis 5/15/01
Phil Hutton, Branch Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division, 7511C

Subject: Review of the ABSTC response to the data call-in (DCI) notice issued on 12/15/99 by the Biopesticides and Pollution Prevention Division (BPPD) of the EPA concerning the potential effects of transgenic corn expressing insecticidal Cry proteins from *Bacillus thuringiensis* (*B.t.* corn) on non-target lepidopteran species, particularly monarch butterflies (*Danaus plexippus*). MRID numbers 45366801 and 45366802.

Background:
On 12/15/99, BPPD issued a data call-in (DCI) for all Bt corn plant-pesticides registered under its FIFRA Section 3(c)2(B) authority, concerning the potential effects of transgenic corn expressing insecticidal Cry proteins from *Bacillus thuringiensis* (*B.t.* corn) on non-target lepidopteran species, particularly monarch butterflies (*Danaus plexippus*) and endangered lepidopteran species. Some of the data required included toxicity testing (on the monarch butterfly and a relative of the endangered Karner blue butterfly), milkweed dispersal, and pollen dispersal.

A response to the DCI was submitted the Agency by Novigen Sciences, Inc on behalf of the Agricultural Biotechnology Stewardship Technical Committee (ABSTC)- Non-Target Organism Subcommittee (formerly the ABSTC Monarch Task Force) (EPA Consortium Number 73207). The ABSTC Non-Target Organism Subcommittee is composed of representatives from the following producers of Bt corn: Aventis CropScience USA LLP (formerly AgrEvo USA Co.), Mycogen Seeds c/o Dow AgroSciences LLC, Monsanto Company, Syngenta Seeds, Inc. (formerly Novartis Seeds, Inc.), and E.I.du Pont de Nemours & Co., Inc.(including Pioneer Hi-Bred International, Inc). Although Aventis CropScience and Pioneer Hi-Bred International are members of the ABSTC Subcommittee on Non-Target Organisms, they are not subject to this

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DCI because they do currently hold Bt corn registrations.
Aventis CropScience CBH351 (Cry9C) and Syngenta Seeds Event 176 (Cry1Ab) were initially subject to this DCI. However the data is no longer necessary because these registrations were voluntarily canceled. In the January 18, 2001 Federal Register, EPA issued a notice of receipt in accordance with section 6(f)(1) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of a request by Aventis CropScience USA LP to cancel their registration of *Bacillus thuringiensis* (B.t.) subspecies *tolworthi* Cry9C and the genetic material necessary for its production in corn. The Agency approved these cancellations effective on February 20, 2001. Event 176 is being voluntarily phased out by Syngenta (EPA Registration No. 66736-1) and Mycogen Seeds c/o Dow AgroSciences (EPA Registration No. 68467-1). Syngenta has ceased production of Event 176 hybrid seed in September 2000 and discontinued harvest of hybrid seed for commercial sale to growers on December 31, 2000. Syngenta's Event 176 registration will expire April 1, 2001 and existing stocks of field corn and popcorn may be distributed until midnight of July 30, 2002. Mycogen Seeds Event 176 registration will expire June 30, 2001 and production of inbred seed for the purpose of producing hybrid seed may continue through June 30, 2001.

The ABSTC relied on several options to address the DCI Data Elements. These include submitting existing data, submitting preliminary data from USDA coordinated research programs, generating data not covered by the USDA programs, and requesting waivers for certain DCI elements. This review follows the required data elements as they were listed in the original DCI. Each element is followed by a short summary of the industry's response, which is followed by the Agency's evaluation and comments on the responses. Whether the submitted responses adequately address the requirement is also indicated.

Discussion:
A study was published in Nature Magazine in May, 1999 by Losey *et al.* that reported a potential risk to monarch butterflies, *Danaus plexippus* Linneaus that feed on Bt corn pollen. In this study, Bt pollen (event Bt11 N4640 Bt corn) was applied to slightly misted milkweeds, *Asclepias syriaca* Linnaeus (Asclepiadaceae) with a spatula in the laboratory. Although the number of grains were not counted, pollen levels in this study were intended to be visually similar to field levels. Results showed that monarch larvae grew slower, gained less weight, and experienced higher mortality when fed on Bt corn pollen. Although this was a worst-case laboratory study, it demonstrates the risk of potential hazard that needed further investigation in the field. In another study, Jesse and Obrycki (2000) observed a decrease in survival of larvae exposed to Event 176 pollen on leaves taken from within the field (80-217 pollen grains/cm^2) compared with those fed leaves taken from outside of the field. Jesse and Obrycki (2000) also reported larval weight gain effects of Bt11 at 135 grains/cm^2. However, these studies should be considered with caution since they did not thoroughly filter additional debris (e.g., anthers) from the pollen that would probably not accumulate on milkweed leaves in the field to a significant degree (Hellmich *et al.* 2001, Pleasants *et al.* 2001, Stanley-Horne *et al.* 2001).

This DCI addresses the potential of monarch exposure to Bt corn pollen in the field and whether pollen densities encountered present a risk to these butterflies. Monarch butterflies potentially

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feed on 14 different species of milkweeds. Seven of these milkweed species are fed on by monarchs in the Corn Belt. Common milkweed (*Asclepias syriaca*) is the predominant species oviposited and fed on by monarchs. Whorled milkweed (*Asclepias verticillata*) may also be an important resource for monarchs (Hartzler and Buhler 2000). Milkweed densities vary and typically depend on the management practices of the habitat.

Milkweeds can be found in a variety of habitats. However, non-agricultural areas are usually undisturbed so more milkweeds are expected. Surveys conducted in Ontario, Maryland, Indiana, Illinois, Iowa, Nebraska, and Kansas showed that more milkweeds occur near the corn field edge, in roadsides, or in non-agricultural areas than within corn fields (Hartzler and Buhler 2000, Oberhauser *et al.* 2000). Roadsides that are mowed will have less milkweed than areas not mowed or tillage practices may affect densities in cultivated fields. It is difficult to control milkweed, particularly when reduce tillage is practiced, because it reproduces vegetatively or by seed and is often found in clumps (Martin and Burside 1984). Herbicides are generally not considered to be effective in controlling milkweeds. However, in some instances, "good" control of milkweeds may be provided by glyphosate, halosulfuron-methyl + dicamba (2,4-D), and nicosulfuron + dicamba.

Some milkweeds occur in and near corn fields, therefore, the proportion of the migrating monarch population that may encounter Bt corn fields was considered. There is potentially 105, 174 square miles (2.73×10^7 hectares) of field corn grown in the U.S. that may provide breeding habitat for monarchs (USDA - NASS 1997). Of this 105,174 square miles, about 26,294 square miles consist of Bt corn fields that may provide breeding sites for monarchs. The edge of corn fields constitutes a very small area of potential monarch breeding habitat. Approximately 0.18% of monarch breeding sites may occur near corn field edges. This is equivalent to 0.11% of all land in this region. It can be concluded that the near edge (within 1 meter of the field edge) of Bt corn fields constitutes a negligible portion of monarch breeding habitat. Approximately 18% of monarch habitat in the central U.S. consists of corn fields (Taylor and Shields 2000) and current approximate acreage of Bt corn is equal to approximately 26,293 square miles (25% of total U.S. corn acreage) or 5.1% of monarch habitat.

Based upon the facts discussed by the ABSTC, it can be assumed that it is not reasonably possible to develop a baseline monarch butterfly population size. However, it is possible to continue surveys such as the one conducted by the Monarch Watch to identify sudden, drastic decreases in the number of monarchs in North America and its overwintering sites in Mexico. The information submitted to the Agency thus far suggests that 50% of monarchs probably pass through the Corn Belt (Taylor *et al.* 1999).

Monarchs feeding on milkweeds in and near Bt corn fields during anthesis will potentially be exposed to Bt pollen. Time to pollination varies among hybrids and regions and is determined according to growing degree units (GDU). GDUs to pollination can be found in commercial seed catalogues. Examples of the approximate number of GDU needed for pollination to occur in different regions are: 1. Fargo, ND = 1130; 2. Madison, WI = 1250; 3. Lincoln, NE = 1370; 4.

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Champaign, IL = 1390; 5. Salisbury, MD = 1400; and 6. Lubbock, TX = 1450. Individual corn tassels typically shed pollen for two to seven days (or longer) and silks on an ear are exposed to pollination for two to three days (Russell and Hallauer 1980, Ritchie *et al.* 1997). A field will shed pollen for up to 15 days depending upon microclimate (Russell and Hallauer 1980).

Corn pollen grains don't disperse far from it's source because they are large (~90 to 100 microns). The majority of corn pollen stays within corn fields and only small quantities disperse beyond 5 m from the field edge. However, pollen levels are higher further into the corn field (e.g., 147.5 grains/cm^2 were found 25 m from the field edge) than close to the edge (e.g., 55.5 grains/cm^2 were found 3 m from the field edge) (Pleasants *et al.* 2001). Raynor *et al.* 1972 found that 63% of corn pollen remained within fields, 88% settled within eight meters of the field edge, and 98% settled within 60 meters. They also determined that there was only 0.2% of pollen deposited at 60 m from the corn field edge. Pleasants *et al.* (2001) found pollen densities at corn field edges were 50% of the level found within the field and densities were greater on milkweed plants within rows than between rows.

It is difficult to report one specific quantity of corn pollen that will be deposited on milkweeds within corn fields and at varying distances from the field edge. Many factors influence pollen deposition and retention on milkweed leaves. Environmental factors such as rain and wind may increase the distance pollen will travel and may decrease the amount of pollen retained on leaves. Plant morphology such as leaf angle will also effect pollen deposition and retention. Upper leaves that are more upright and exposed to environmental factors retain less pollen than middle and lower leaves on the milkweed plant (Pleasants *et al.* 2001).

Pleasants *et al.* (2001) found that levels of corn pollen deposition on milkweed leaves are influenced by wind, wind direction, rainfall, plant architecture and the time period when pollen was sampled. In some instances, weather conditions such as thunderstorms and updrafts carry some pollen grains further than usual (Emberlin 1999). However, wind, rain, and other environmental factors will probably remove most of the pollen deposited on milkweed leaves (Pleasants *et al.* 2001). Rainfall has been shown to remove most (86 - 92%) of the pollen from milkweed leaves, thus potentially reducing the length of monarch exposure to Bt pollen (Pleasants *et al.* 2001, Stanley-Horne *et al.* 2001). The level of exposure of monarch larvae to Bt pollen carried to milkweed plants on exoskeleton of adults is minimal. If pollen were to adhere to monarch adults and dislodge on milkweeds, quantities would not be great enough to adversely affect larvae feeding on these milkweeds. Since Bt Cry protein must be ingested and will not harm monarchs by contacting it's exoskeleton, there is minimal risk posed from monarchs transporting pollen among milkweed plants.

Monarchs will only be exposed to Bt while it remains biologically active in pollen. Microbial enzymes, secondary plant compounds, extremes in pH, ultraviolet light, wind and rain are known to degrade Bt proteins in microbial sprays. The insecticidal activity in Bt microbial sprays has been shown to break down rapidly for two days after application and is practically nonexistent four days post application (Gelernter 1990). These factors probably also affect the insecticidal activity of Bt expressed in pollen. Head and Brown (1999) only found biological activity of MON 810 in fresh pollen. Laboratory assays showed that MON 810 activity was not detectable in pollen after seven days (Head and Brown 1999). The biological activity of Bt proteins probably decreases more rapidly in the field where it is exposed to elements such as ultraviolet light than in the laboratory. Therefore, the Bt may breakdown more rapidly than seven days under field conditions.

Monarch ovipositional and feeding behavior will also contribute to the level of milkweed pollen larvae will encounter. Surveys have shown that monarchs prefer to oviposit single eggs on the underside of milkweed leaves on young, tender tissue (Urquhart 1960, Borkin 1982, Pleasants *et al.* 2001). However, females may lay more than one egg per plant and may oviposit on the top of leaves, on stalks or flowers (Borkin 1982). Age of plant tissue is probably the most important influence on monarch ovipositional preference. Female monarchs prefer to oviposit on young tender plant tissue (Urquhart 1960, Borkn 1982). Neonate larvae begin feeding near the area where eggs were laid which is typically the underside of leaves. As larvae mature, they may feed through leaves, on top of leaves, or on leaf veins (Urquhart 1960).

Results vary regarding monarch preference, avoidance, or indifference to ovipositing on milkweeds in corn fields (Losey *et al.* 2000, Oberhauser *et al.* 2001). In the laboratory, Losey *et al.* (2000) showed that monarchs either do not show a preference for milkweeds with or without corn pollen dusted on them, or they avoid pollen dusted milkweeds. Field surveys conducted by Oberhauser *et al.* (2001) and Stanley-Horne *et al.* (2001) found monarch eggs on milkweeds dusted with pollen in and near corn fields. In some instances monarchs may prefer to oviposit on milkweeds occurring within corn fields (Oberhauser *et al.* 2001). Although milkweed densities are generally higher in nonagricultural habitats, surveys conducted in Minnesota, Wisconsin, and Iowa suggest that monarchs will oviposit in corn fields 45 to 107 times more often than in nonagricultural habitats (Oberhauser *et al.* 2001).

Oberhauser *et al.* (2001) and Pleasants *et al.* (2001) showed that monarchs do occur on milkweeds in the field during pollen shed. The Oberhauser *et al.* (2001) study showed considerable overlap between the peak of the migratory monarch generation and pollen shed in Minnesota and Ontario. In Iowa and Maryland, the final generation of monarchs peaked prior to pollen shed. According to models developed by Calvin *et al.* (2000), overlap of monarch larval occurrence and corn pollination is negligible in southern and central parts of the Corn Belt, but there is up to 75% overlap in the northernmost area of the Corn Belt. This means that 0 to 5% of monarchs will be exposed to Bt corn pollen in most of the Corn Belt and 10% exposure will occur in the northern region. In general, the Calvin *et al.* (2000) model showed that the degree of co-occurrence generally increased as latitude or elevation increased.

Since monarch eggs and larvae were found on milkweed plants in the northern fields when pollen was present on leaves (Oberhauser *et al.* 2001), there is a high probability that some monarchs will encounter Bt corn pollen. It can also be assumed that monarch larvae will consume both Bt or non-Bt pollen if it is encountered (Hellmich *et al.* 2001, Oberhauser *et al.* 2001). Laboratory

(Hellmich *et al.* 2001, Losey *et al.* 2001) and field studies (Oberhauser *et al.* 2001) demonstrated that monarchs will not avoid feeding on plants dusted with Bt or non-Bt corn pollen. Since eggs and larvae were found on milkweed plants naturally dusted in corn pollen in the field, it can be assumed that foraging monarchs will not avoid pollen dusted plants nor do they avoid corn fields.

Since it has been established that monarchs will encounter and feed on Bt pollen in the field, it is important to know the LC_{50}. The LC_{50}s for the various monarch larval stages fed purified trypsin resistant core of various Bt Cry proteins were found to be: a. 1st instars = 3.29 ng Cry1Ab/mL treated artificial diet; b. 2nd - 3rd instars = 35.1 ng Cry1Ab/mL treated artificial diet; c.. 3rd - 4th instars = >100 ng Cry1Ab/mL treated artificial diet (Hellmich *et al.* 2001). Since monarchs are probably exposed to varied amounts of Bt in the field and the activity of Bt in pollen declines over time, the LC_{50}s determined from this laboratory experiment are probably higher than exposure in the field.

Cry 1Ab is only found in pollen of the currently registered Bt field corn hybrids (MON 810 and Bt11) in trace quantities (<0.09 μg/g dry wt. pollen). First instar monarch larvae are the most sensitive stage Bt and young larvae are generally more susceptible than older larvae (Hellmich *et al.* 2001, Stanley-Horne *et al.* 2001). First instar larvae feeding on milkweed leaves naturally dusted with pollen in the field resulted in no observable effects of MON 810 and Bt11 on survival and fitness of monarchs. Five independent surveys were conducted during the 2000 growing season in Iowa, Maryland, and Ontario. The highest average corn pollen density monitored in the field was 586 grains/cm^2 found three meters inside a Bt11 sweet corn field (Stanley-Horne *et al.* 2001). It is not surprising that the highest pollen densities were found in a Bt sweet corn field since sweet corn produces more pollen per plant than field corn. There was no difference in densities of Bt and non-Bt pollen found on milkweed leaves (Stanley-Horne *et al.* 2001). In one corn field in Iowa, Pleasants *et al.* (2001) found 900 pollen grains/cm^2. Extremely high pollen levels (250 - 2000 grains/cm^2 for MON 810 and 150 - 4000 grains/cm^2 for Bt11) were fed to first instar monarch larvae in a controlled environment and resulted in no significant effects on larval weight (Hellmich *et al.* 2001). From this data, it can be concluded that the NOEL (No observable effect level) for MON810 is >2000 grains/cm^2 and for Bt11 is >4000 grains/cm^2 which is greater than levels that occur under natural field conditions.

The DCI also addressed the potential of Karner blue butterflies being adversely affected by Bt corn pollen in the field. Since endangered species such as the Karner blue cannot be directly tested and susceptibility of the Karner blue is not necessarily equivalent to other species from the genus Lycaeides, determining an LC_{50} is difficult to impossible. Herms *et al.* (2001) demonstrated that Karner blue butterflies are susceptible to Cry1Ab through the application of a Bt microbial spray (Foray 48B). Therefore, it can be assumed that the Karner blue is susceptible to MON810 and Bt11 and will be adversely affected if toxic levels of pollen are ingested. However, the Karner blue is probably no more sensitive to Cry1Ab than monarch butterflies and will not consume toxic levels of Bt in the field. In addition, Karner blues feed on wild lupines which occur in pine barrens and oak savannahs and do not typically occur near corn fields. Therefore, Karner blue butterflies are probably not adversely affected by MON 810 and Bt11 in the field. However, wild lupines may occur near some corn fields in areas such as Wisconsin where the weed grows in fallow fields and promotes Karner blue establishment. If Bt corn is planted in these fields where wild lupines have been established in following years, exposure to MON 810 and Bt11 may occur. Therefore, the Agency is initiating a consultation with the Fish and Wildlife Service (FWS) to determine potential impacts of Bt corn on Karner blue butterflies.

Conclusions:
Studies conducted since this DCI was initiated have shown that monarch larvae feeding on corn pollen expressing MON 810 or Bt11 do not demonstrate observable adverse effects on survival, weight, or other fitness parameters (e.g., developmental change, weight gain, percent survival to pupation, pupal weight (mg), percent emergence from pupae, adult weight (mg), or adult wing length) (Stanley-Horne *et al.* 2001). Risk from other factors such as destruction of overwintering habitat, weather, predators, pesticides, physiological stress, and human activity (Taylor 1999) are a much greater and more widespread threat to monarch populations than the use of Bt corn. The risk Bt corn pollen poses to monarchs is also probably relatively less than other significant risk factors especially insecticide use (Stanley-Horne *et al.* 2001). The potential reduction of insecticide use that may result from planting Bt corn will most likely benefit monarch populations as well as other beneficial insect, especially in and sweet corn production.

Data submitted by the ABSTC demonstrates that levels of MON 810 and Bt11 corn pollen toxic to monarchs will probably not occur under natural field conditions. The highest average corn pollen densities monitored in the field were 586 grains/cm^2 in Maryland (Stanley-Horne *et al.* 2001) and 900 grains/cm^2 found in one Iowa corn field (Pleasants *et al.* 20001). Research conducted in response to this DCI showed that MON810 and Bt11 corn pollen densities of < 1600 grains/cm^2 are not toxic to monarchs (Hellmich *et al.* 2001). In a worst case scenario, pollen deposition when no rainfall occurred was approximately 1400 grain/cm^2 (Pleasants *et al.* 2001). In another study, extremely high pollen levels (250 - 2000 grains/cm^2 for MON 810 and 150 - 4000 grains/cm^2 for Bt11) were fed to first instar monarch larvae in a controlled environment and resulted in no significant effects on larval weight (Hellmich *et al.* 2001). From this data, it can be concluded that the NOEL for MON 810 is >2000 grains/cm^2 an for Bt11 is >4000 grains/cm^2 which is greater than levels that occur under natural field conditions. Studies have shown that the order of monarch sensitivity to Cry proteins is Cry1Ab > Cry1Ac > Cry9C > Cry1F. Only pollen from Event 176 corn has been shown to adversely affect growth, fitness, and mortality of monarch butterflies (Losey *et al.* 1999, Jesse and Obrycki 2000, Stanley-Horne *et al.* 2001). However, this does not create a concern for monarchs since Event 176 corn comprises less than 2% of U.S. corn acreage and will no longer be sold after the 2003 growing season.

The ABSTC suggested that inbred corn lines are homozygous for the *Bt* gene and are expected to express twice the level of Bt as hybrids which are hemizygous for the *Bt* gene. Since inbreds are only found in small, isolated fields used for seed production that are kept clean of weeds and occur on less than 1% of the corn acreage, monarch exposure to inbred corn pollen is expected to be minimal. Therefore, pollen expressing Bt from inbred lines do not present a greater risk to monarchs that hybrid corn pollen. It is, therefore, acceptable that the ABSTC submitted data on

Bt hybrids only. Data requested for inbreds is waived.

Based on the lack of toxicity from the pollen of MON 810 and Bt11, the ABSTC is requesting a waiver from requiring the following information in the text of informational material provided to Bt corn growers: "The potential for non-target species (e.g., monarch butterfly) to be affected by Bt corn pollen remains under study. As an interim measure, the EPA is encouraging growers to place the non-Bt corn refuge between Bt corn and habitats such as prairies, forests, conservation areas, and roadsides." The ABSTC also believes there is a greater concern of insecticide exposure if refuges are planted along the field edge since pest pressure is higher along field edges leading to a greater potential of chemical treatment and because drift from airplane applications may reach non-treated field edges. Since data submitted to the Agency in response to this DCI showed that Bt corn pollen in the field does not pose a risk to monarch butterflies, it is acceptable to waive the requirement of including the above statement on Bt corn grower information guides.

DATA EVALUATION REPORT

REVIEWED BY:	Robyn Rose, Entomologist RR 5/15/01 Biopesticides and Pollution Prevention Division
SECONDARY REVIEWER:	Zigfridas Vaituzis, Ph.D., Microbiologist ZV 5/15/01 Biopesticides and Pollution Prevention Division
STUDY TYPE:	Response to the EPA Bt corn generic data call-in regarding effects on monarch butterflies
MRID NOs.:	45366801 and 45366802
EPA CONSORTIUM NO.:	73207
SPONSOR:	Agricultural Biotechnology Stewardship Technical Committee, Non-Target Organism Subcommittee
SUBMITTED BY:	Novigen Sciences Inc., 1730 Rhode Island Ave, NW, Ste 1100, Washington, DC 20036
TEST MATERIAL:	*Bacillus thuringiensis* subspecies *kurstaki* events MON 810 and Bt11 as expressed in field corn and sweet corn
AUTHOR:	Edwin F. (Rick) Tinsworth
STUDIES DATED:	March 29, 2001

DCI Data Element 1:

(1) Determine and report (in square miles) the total land mass in North America that contains milkweed and monarchs vs. the total amount of land at the edge of corn fields where milkweed could be exposed to Bt corn pollen.

Classification: Acceptable

Summary of ABSTC's Response:

Since field corn production in the western U.S. is a very small fraction of total acres grown nationally, only the eastern monarch population (east of the Rocky mountains in the U.S. and Canada) was considered. Breeding areas of the eastern population were considered to occur from 32° to 48° North Latitude and from 95° West longitude to the Atlantic Coast. After one season of sampling, Wassenaar and Hobson (1998) found that 50% of monarchs occur in the Corn Belt (Nebraska to Ohio) over approximately 750,000 square miles (1.9×10^8 hectares). However, Taylor and Shields (2000) determined that monarch summer breeding area in the U.S. ranged over 1,543,750 square miles (4×10^8) with 1.87×10^8 hectares occurring in the Corn Belt. Therefore, approximately 10.5% of monarch breeding area in the U.S. is comprised of corn fields and 18.3% of the Corn Belt consists of corn fields (Taylor and Shields 2000).

A total of approximately 105, 174 square miles (2.73×10^7 hectares) of field corn are grown in the U.S. Field corn in the U.S. is grown in Delaware (0.2% of total U.S. corn acres), Iowa

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(16.6% of total U.S. corn acres), Illinois (15.3% of total U.S. corn acres), Indiana (7.8% of total U.S. corn acres), Kansas (3.6% of total U.S. corn acres), Kentucky (1.6% of total U.S. corn acres), Maryland (0.6% of total U.S. corn acres), Michigan (3.0% of total U.S. corn acres), Minnesota (8.9% of total U.S. corn acres), Missouri (3.5% of total U.S. corn acres), North Dakota (0.8% of total U.S. corn acres), Nebraska (11.9% of total U.S. corn acres), New York (0.8% of total U.S. corn acres), Ohio (4.8% of total U.S. corn acres), Pennsylvania (1.4% of total U.S. corn acres), South Dakota (4.5% of total U.S. corn acres), Virginia (0.5% of total U.S. corn acres), Wisconsin (4.1% of total U.S. corn acres), West Virginia (0.1% of total U.S. corn acres) (USDA - NASS 1997).

The total amount of land at the edge of corn fields was determined from information on the size of the farm, number of fields per farm, field dimensions, and width of the field edge. The average farm being cultivated for field corn seed, grain, or silage in the monarch breeding area is 146 acres (59.1 ha) (USDA - NASS 1997). The ABSTC found that 1% o the planted field area consists of a one-meter field edge margin which is equivalent to approximately 1052 square miles (272,611 hectares). Therefore, 0.18% of monarch breeding area (570,045 square miles or 1.5×10^8 ha) may occur near corn field edges; this is equal to 0.11% of all land area in this region.

Approximately 25% of the field corn planted in the Midwest is Bt. Therefore, Bt corn is planted on approximately 26,294 square miles which is equivalent to about 4.6% of the monarch summer breeding area described by Taylor and Shields (2000). Bt corn fields plus the surrounding one meter of field edge is equal to about 28,293 square miles or5.1% of monarch habitat in the Corn Belt. The highest possible adoption rate of Bt corn is 80% of the field corn acreage in the Corn Belt. According to ABSTC, with 80% adoption, about 16.1% (86,395 square miles) of monarch habitat in the Corn Belt would be Bt and field edge.

<u>BPPD Conclusions</u>:

The edge of corn fields constitutes a very small area of potential monarch breeding habitat. Only 0.18% of monarch breeding sites may occur near corn field edges. This is equivalent to 0.11% of all land in this region. Current approximate acreage of Bt corn is equal to approximately 28,293 square miles or 5.1% of monarch habitat. It can be concluded that the near edge (with 1 meter of the field edge) of Bt corn fields constitutes a negligible portion of monarch breeding habitat.

However, current research has shown that milkweeds occurring within corn fields may provide important breeding habitat for monarchs (Oberhauser *et al.* 2001). Therefore, there is potentially 105, 174 square miles (2.73×10^7 hectares) of field corn grown in the U.S. that may provide breeding habitat for monarchs. Of this 105,174 square miles, about 26,294 square miles consist of Bt corn fields that may provide breeding sites for monarchs.

DCI Data Element 2:

(2) Determine and report what species of milkweed monarchs feed on.

Classification: Acceptable

<u>Summary of ABSTC's Response</u>:

Of the fourteen species of milkweed that monarchs feed on in North America (Erickson 1973), common milkweed (*Asclepias syriaca*) is the dominant food source. Studies conducted by Dr. Orley Taylor (Kansas State University; Director of Monarch Watch) demonstrate that common milkweed has the highest rate of monarch oviposition. However, Dr. Robert Hartzler (Iowa State University) conducted a survey in Iowa in 1999 that suggested that monarchs may prefer whorled milkweed. Since monarchs prefer young milkweeds, the difference in plant maturity may explain why monarchs are found on whorled milkweed which mature later than common milkweed (Dr. Orley Taylor personal communication with ABSTC).

According to Monarch Watch (2000), 90% of the monarchs passing through the Corn Belt will feed on the following seven common milkweed species: 1. *Asclepias syriaca* (common milkweed), 2. *Asclepias incarnata* (swamp milkweed), 3. *Asclepias speciosa* (showy milkweed), 4. *Asclepias tuberosa* (butterfly milkweed), 5. *Asclepias verticillata* (whorled milkweed), 6. *Asclepias viridis* (spider milkweed), and 7. *Cynanchum laeve* (blue vine milkweed).

<u>BPPD Conclusions</u>:

Monarch butterflies potentially feed on 14 different species of milkweeds. Seven of these milkweed species are fed on by monarchs in the Corn Belt. Common milkweed (*Asclepias syriaca*) is the predominant species oviposited and fed on by monarchs. Whorled milkweed (*Asclepias verticillata*) is also an important resource for monarchs.

DCI Data Element 3:

(3) Determine and report what percentage of milkweed in the Corn Belt is found in row crop areas *vs.* roadsides, pastures and other non-row crop areas.

Classification: Acceptable

<u>Summary of ABSTC's Response</u>:

Since perennial weeds are typically more abundant in undisturbed habitats (Buhler 1995), non-cropped areas are expected to have more milkweeds than cultivated land such as corn and soybean fields (Hartzler and Buhler 2000). Generalizations regarding milkweed abundance and distribution is difficult since changes in land use will have an effect in different areas.

Management of crop and non-crop areas will effect milkweeds. Impacts to milkweeds occur from farming practices such as tillage, herbicide applications, and cropping choices as well as non-crop management such as mowing roadside ditches. In some areas such as Ontario, Canada, milkweed is considered a noxious weed and is aggressively managed.

Surveys conducted by Cramer and Burnside (1982) found common milkweed occurring in soybeans, oats, sorghum, railroads, fallow areas, roadsides, corn, wheat, pastures, and alfalfa fields. Hartzler and Buhler (2000) conducted a similar survey in Iowa cropland and adjacent areas and found milkweeds in roadsides, corn fields, soybeans, pasture, waterways and terraces, and conservation reserves. Both of these surveys showed a >50% milkweed infestation in roadsides and fallow/conservation reserve fields and relatively lower milkweed infestations in corn fields and pastureland. This study showed that the number of milkweed patches are approximately seven times greater along roadsides than in cornfields, soybeans, or pastures. However, since 78% of Iowa's land area is corn, soybean, or pasture, (Tiffany and Miller 1999) a significant portion of milkweeds will occur in these cultivated fields.

A relative assessment of milkweed infestations in a particular land use site is useful, however, a better understanding of milkweed densities within the infested areas would aid in understanding milkweed distribution. In Ontario, Canada corn is grown on 0.7% of the land (~1.7 million acres in 2000) and is greatest in the southwest where milkweed densities were found to be 115.5-fold higher in uncultivated areas than in corn (Sears *et al.* 2000). In southwestern Ontario, milkweed densities were 80 ramets/ha in corn fields and 9,240 ramets/ha in non-agricultural areas (Oberhauser *et al.* 2001). Therefore, it can be concluded that most of the milkweed in Ontario occurs outside of corn fields.

Oberhauser *et al.* (2000) reported milkweed densities in Minnesota, Wisconsin, and Maryland corn fields. However, the results of this study were not considered in response to this data element because formal surveys were not taken and, therefore, results from this study do not represent milkweed densities in the Corn Belt.

<u>BPPD Conclusions</u>:

It is difficult to quantify the exact level of milkweeds occurring in an area since many factors effect densities such as farming practices (e.g., tillage, herbicide applications, and cropping choices) as well as non-crop management (e.g., mowing roadside ditches). It can be concluded that relatively more milkweeds occur in non-agricultural areas such as roadsides, fallow areas, and waterways than in cultivated cropland such as corn fields. However, since corn fields constitute a large portion of land in the Corn Belt, the number of milkweeds found in this area is probably significant.

DCI Data Element 4:

(4) Provide surveys of corn fields in representative corn growing states to determine how much milkweed is in the fields.

4(a) Determine and report whether milkweeds are closer, farther or at random distance to corn.

4(b) Provide data on the relative abundance of milkweed in the corn field pollen shadow versus areas further than 60 meters away from corn fields.

4(c) Determine and report whether herbicides are effective in corn fields in eliminating milkweeds. If so, determine which herbicides are most effective.

Classification: Acceptable

Summary of ABSTC's Response:

4(a) Determine and report whether milkweeds are closer, farther or at random distance to corn.

4(b) Provide data on the relative abundance of milkweed in the corn field pollen shadow versus areas further than 60 meters away from corn fields.

Hartzler and Buhler (2000) found milkweeds infesting 46% of the Iowa corn fields randomly sampled. Surveyed corn fields averaged 0.7 milkweed patches (stems ≤ 1 m apart) per hectare over 30 m^2 per hectare. A 3.4-fold increase was found in Iowa roadsides than in corn fields and a 7.1-fold increase in milkweed density in non-agricultural areas than corn fields (Hartzler and Buhler 2000). Generally, milkweeds are expected to be less dense in corn fields than in roadsides because of management practices in corn fields and typically higher infestation of perennial weeds in undisturbed areas.

Milkweed densities in randomly surveyed corn fields in Ontario, Canada were 0.0008 milkweed/m^2 or 80 milkweed ramets per hectare. In Ontario, milkweed densities were 115.5-fold lower in corn fields than in non-agricultural areas (Oberhauser *et al.* 2000). Oberhauser *et al.* (2000) reported milkweed densities in Minnesota, Wisconsin, and Maryland corn fields. However, surveyed fields were not necessarily representative of milkweed density in typical corn fields in Minnesota, Wisconsin, and Maryland because only fields that had at least 10 milkweed ramets per hectare were sampled. The five fields surveyed in Minnesota and Wisconsin had an average of 0.525 milkweed/m^2 within ten meters of corn field edges which represents 1.8-fold more milkweeds in the field edge than within the field (Oberhauser *et al.* 2000). Surveys conducted by Cramer and Burnside (1982) in Nebraska found common milkweed occurring in 36% of the corn fields observed while driving by.

Surveys conducted in 1999 in Maryland, Indiana, Illinois, Iowa, Nebraska, and Kansas showed milkweed occurring in roadsides next to corn fields in 15% (Illinois) to 41% (Iowa) of the samples. The occurrence of milkweeds in roadsides adjacent to corn fields may vary due to management practices (e.g., mowing) or between seasons. Most milkweeds surveyed near corn field edges in Indiana, Maryland, and Illinois occurred within one to three meters form corn fields and declined toward roadsides (probably due to mowing). Milkweeds in Iowa were uniformly distributed from one to 20 meters from the corn field edge (Isenhour *et al.* 2000).

Since pollen levels were negligible beyond two meters from the corn field edge (Pleasants *et al.* 2001), the ABSTC suggested that distribution of milkweeds further than two meters from the field edge are not relevant when considering Bt corn pollen effects. The ABSTC also stated that "the corn pollen shadow relevant to questions of milkweed distribution and corn pollen interception is much more restricted than 60 meters."

BPPD Conclusions:

Surveys conducted in Ontario, Maryland, Indiana, Illinois, Iowa, Nebraska, and Kansas showed that more milkweeds occur outside of corn fields near the field edge, in roadsides, or in non-agricultural areas (Hartzler and Buhler 2000, Oberhauser *et al.* 2000). The density of milkweeds vary and typically depend on the management practices of the habitat. For instance, roadsides that are mowed will have less milkweed than areas not mowed or tillage practices may affect densities in cultivated fields. Since non-agricultural areas an usually undisturbed, more milkweeds are expected.

4(c) Determine and report whether herbicides are effective in corn fields in eliminating milkweeds. If so, determine which herbicides are most effective.

It is difficult to control milkweed, particularly when reduce tillage is practiced, because it reproduces vegetatively or by seed and is often found in clumps (Martin and Burside 1984). Although milkweed is not typically targeted for pest control, herbicides and cultural practices may be used to manage it (Yenish *et al.* 1997). Herbicide efficacy against milkweeds may vary from region to region and year to year because weather, crop/weed phenology, timing of application and environmental effects. Even those herbicides that provide "good" control do not eliminate milkweeds from corn fields.

Of the 30 registered herbicides to control milkweed © & P 2000), none are considered to provide excellent control of milkweed. Glyphosate, halosulfuron-methyl + dicamba (2,4-D), and nicosulfuron + dicamba have been reported to provide "good" control of milkweed. However, these herbicides are rarely or never used in corn or soybeans. The following table summarizes the percent acreage of corn and soybeans sprayed with herbicides that are considered "good" control of milkweed.

Herbicide	% Corn Acres Sprayed	% Soybean Acres Sprayed
2,4-D	12	11
dicamba	25	0
nicosulfuron	11	0

14/4

Herbicide	% Corn Acres Sprayed	% Soybean Acres Sprayed
halosulfuron	2	0
glyphosate	9	27

(NCFAP 19997). It should be noted that glyphosate use will probably increase since the introduction of glyphosate-resistant soybeans.

BPPD Conclusions:

Herbicides are not considered to be effective in controlling milkweeds. It is probably difficult to control milkweed because it spreads by underground roots. Herbicides that may provide "good" control of milkweeds are glyphosate, halosulfuron-methyl + dicamba (2,4-D), and nicosulfuron + dicamba

DCI Data Element 5:

(5) Determine and report what is the relationship between monarch colonization of milkweeds and distance to corn.

5(a) Determine and report the distribution of monarch eggs and larvae on milkweeds relative to corn fields.
5(b) Quantify and report the pollen on milkweed leaves within the pollen shadow and up to 60 meters from the edge of Bt corn fields.
5(c) Provide the distances from the edge of corn field at which LD50 concentrations of Bt pollen are found for each Bt corn hybrid.

Classification: Acceptable

Summary of ABSTC's response:

5(a) Determine and report the distribution of monarch eggs and larvae on milkweeds relative to corn fields.

Studies have shown that monarchs either do not show a preference for milkweeds with or without corn pollen dusted on them, or they avoid pollen dusted milkweeds (Losey *et al.* 2000). Monarchs will oviposit on milkweeds growing in Bt and non-Bt cornfields (Oberhauser *et al.* 2001). In surveys conducted in Maryland and Wisconsin, monarchs did not show an ovipositional preference for milkweeds found in corn fields, near field edges, other agricultural fields, or non-agricultural areas. However, in surveys conducted in Minnesota and Wisconsin, more monarchs oviposited on milkweeds found in corn fields than non-agricultural habitats and were not affected when Bt or non-Bt pollen was present (Oberhauser *et al.* 2001). Although all of the surveys showed milkweeds were shorter in corn fields than non-agricultural areas, new attractive growth occurred in the upper Midwest due to plowing, mid-season cultivation, and/or fertilizing (Oberhauser *et al.* 2001).

Therefore, the ABSTC concluded that female monarchs either do not avoid ovipositing in corn fields or prefer to oviposit in corn fields. However, they may avoid ovipositing on milkweeds covered with high pollen densities.

5(b) Quantify and report the pollen on milkweed leaves within the pollen shadow and up to 60 meters from the edge of Bt corn fields.

The quantity of corn pollen on milkweed leaves is affected by the proximity of milkweeds to corn fields, milkweed architecture and morphology, pollen movement away from the field, and environmental factors. Although the ABSTC defines the pollen shadow as the area adjacent to the field edge to 60 m away, they recognized that the majority of pollen is deposited within the field to 5 m out and drops exponentially with further distances from the field edge. However, Pleasants *et al.* 2001 found only 10 grains/cm^2 on milkweeds 5 m from the corn field edge. Even at higher wind speeds (e.g., one to 10 meters/second), most of the corn pollen remains in the field. Low concentrations of pollen may move further distances from corn fields at high wind speeds (Weiss and Reid 1999). Although pollen densities are higher within corn fields than outside, pollen densities were greater deeper in the field than close to it's edge (Pleasants *et al.* 2001). Raynor *et al.* 1972 found that 63% of corn pollen remained within fields, 88% settled within eight meters of the field edge, and 98% settled within 60 meters. There was only 0.2% of pollen levels deposited at 60 m from the corn field edge than within fields. Corn pollen grains don't disperse far from its source because they are large (~90 to 100 microns). In some instances, weather conditions such as thunderstorms and updrafts carry some pollen grains further than usual (Emberlin 1999).

A two year survey was conducted in Iowa, Ontario, and Maryland that evaluated 5000 leaf samples from more than 100 corn fields collected during or at the end of pollen-shed. Results indicated that 65.7 grains/cm^2 to 231.4 grains/cm^2 can be expected in corn fields during 50-100% pollen-shed. In one case in Iowa, pollen density reached 425.6 grains/cm^2. In addition to mean pollen densities, this survey evaluated the distribution of pollen densities. This survey showed that <500 grains/cm^2 occurred inside corn fields 96.1% of the time, 1000 grains/cm^2 occurred in fields 99.3% of the time and 1600 grains/cm^2 was never reached (occurred 0% of the time) within corn fields (Pleasants *et al.* 2001). On average, 119.6 grains/cm^2 were found within corn fields, 63.1 grains/cm^2 occurred at the corn field edge, 35.4 grains/cm^2 were collected one meter from the edge, 14.2 grains/cm^2 were found at two meters and 8.1 grains/cm^2 occurred at four to five meters from the edge (Pleasants *et al.* 2001).

Levels of corn pollen deposition on milkweed leaves are influenced by wind, wind direction, rainfall, time period pollen was sampled. In Pleasants *et al.* (2001) study, approximately 40% of pollen in the air was retained on leaves. Most of the pollen (86%) was removed from leaves after one 1.9 cm rainfall. Location of milkweed plants within corn fields or location of leaves on milkweeds also effect the number of deposited pollen grains. For example, 176.6 grains/cm^2

were found on milkweeds within corn rows and 118.9 grains/cm^2 were deposited on plants between rows. Pollen levels within corn fields were higher 25 meters from the edge (147.5 grains/cm^2) than three meters from the edge (55.5 grains/cm^2) of the corn field. In addition, significantly more pollen was retained on middle and lower leaves than top leaves on the milkweed plant because of rainfall and , wind and/or plant movement. The increase pollen level on lower leaves may also be due to leaf orientation; upper leaves are situated more upright and are smaller than lower leaves (Pleasants *et al.* 2001).

5(c) Provide the distances from the edge of corn field at which LD50 concentrations of Bt pollen are found for each Bt corn hybrid.

Monarch butterflies feeding on 1600-2000 pollen grains/m^2 of MON 810 and 4000 pollen grains/cm^2 of Bt11 were not adversely affected in a laboratory study (Hellmich *et al.* 2001). Although exact LC_{50} values have not been determined, it can be assumed that they're >2000 grains/cm^2 for MON810 and >4000 grains/cm^2 for Bt11. Of the >5000 individual leaves collected around corn fields and 1320 samples taken inside corn fields, pollen density on milkweeds never exceeded 1600 grains/cm^2 (Pleasants *et al.* 2001). No effects on mortality, weight, size, or developmental time were found in the field when first instar monarch larvae fed on milkweed leaves with up to 586 Bt pollen grains/cm^2 and third instars fed on 97 grains/cm^2 of Bt 11 (Stanley-Horne *et al.* 2001). Additional field studies showed that 23 to 67 pollen grains/cm^2 of Event 176 significantly reduced weight gain, growth, and/or survival of first instar larvae and 12-13 grains/cm^2 reduced larval weight (Stanley-Horne *et al.* 2001). Therefore, it can be concluded that monarchs will develop normally on milkweeds within on near MON 810 and Bt11 corn fields.

BPPD Conclusions:

Results vary regarding monarch preference, avoidance, or indifference to ovipositing on milkweeds in corn fields (Losey *et al.* 2000, Oberhauser *et al.* 2001). However, it can be concluded that in some instances monarchs prefer to oviposit on milkweeds occurring within corn fields (Oberhauser *et al.* 2001). The majority of corn pollen stays within corn fields and only small quantities disperse beyond 5 m from the field edge. However, pollen levels are higher further into the corn field (e.g., 147.5 grains/cm^2 were found 25 m from the field edge) than close to the edge (e.g., 55.5 grains/cm^2 were found 3 m from the field edge) (Pleasants *et al.* 2001).

Corn pollen grains don't disperse far from it's source because they are large (~90 to 100 microns). In some instances, weather conditions such as thunderstorms and updrafts carry some pollen grains further than usual (Emberlin 1999). However, wind, rain, and other environmental factors will probably remove most of the pollen deposited on milkweed leaves (Pleasants *et al.* 2001). Raynor *et al.* 1972 found that 63% of corn pollen remained within fields, 88% settled within eight meters of the field edge, and 98% settled within 60 meters. There was only 0.2% of pollen deposited at 60 m from the corn field edge than within fields. Research conducted in response to this DCI showed that MON810 and Bt11 corn pollen densities of < 1600 grains/cm^2

are not toxic to monarchs. The maximum level of corn pollen density monitored in the field was 586 grains/cm^2 (Stanley-Horne *et al.* 2001). Data submitted by the ABSTC demonstrates that levels of MON 810 and Bt11 corn pollen toxic to monarchs will probably not occur under natural field conditions.

It is difficult to report one specific quantity of corn pollen that will be deposited on milkweeds within corn fields and at varying distances from the field edge. Many factors influence pollen deposition and retention on milkweed leaves. Environmental factors such as rain and wind may increase the distance pollen will travel and may decrease the amount of pollen retained on leaves. Plant morphology such as leaf angle will also effect pollen deposition and retention. Upper leaves that are more upright and exposed to environmental factors retain less pollen than middle and lower leaves on the milkweed plant (Pleasants *et al.* 2001).

DCI Data Element 6:

(6) Determine and report the LD50s for the Cry protein in your Bt corn active ingredient(s) for a) monarch larvae and for b) larvae of a relative of the endangered Karner blue butterfly. The Karner blue butterfly relative tested must be from the genus Lycaeides, such as the Northern Blue butterfly (*Lycaeides idas*). If it is not feasible to test a butterfly from the genus Lycaeides, then you must provide justification regarding why such testing is not feasible and test a butterfly from a genus within the family Lycaenidae. The Karner blue butterfly must not be tested.

Classification: Acceptable

Summary of ABSTC's response:

The Cry1F protein has no detectable impact on monarch larvae, while the Cry9C protein is about 230-fold lower in toxicity than the Cry1Ab protein, and 23-fold lower than the Cry1Ac protein. Therefore, the order of sensitivity is Cry1Ab > Cry1Ac > Cry9C > Cry1F.

Monarch larvae were fed purified trypsin resistant core of various Bt Cry proteins (Cry1Ab, Cry1Ac, Cry9C, and Cry1F) incorporated in diet to determine LC_{50} and EC_{50} values in ng/mL diet. Laboratory assay results for Cry1Ab after seven days of exposure are reported in the following table.

Instar (N)	LC_{50} (95% C.I.)	EC_{50} (95% C.I.)
1st (318)	3.29 (2.19-4.76)	0.76 (0.64-0.90)
2nd - 3rd (141)	35.1 (30-100)	9.60 (6.01-15.06)
3rd - 4th (125)	> 100 (-)	18.3 (9.4-40.3)

(Hellmich *et al.* 2001).

LC_{50}s for third and fourth instars were 30 times greater than first instars and second and third instar's LC_{50} was 11 time greater than first instars. The Cry1Ab no observable effect concentration (NOEC) was reported as ≤0.3 ng/mL diet (Hellmich *et al.* 2001).

In nature, monarchs are not expected to get uniformly distributed doses of Bt as is observed in the laboratory. Unlike feeding on diet in the laboratory, monarchs would probably ingest varied amounts of Bt in the field and also have the opportunity to avoid feeding on Bt by feeding around pollen grains. Bt activity in pollen is also expected to decline over time in the field. Therefore, levels of Cry1Ab ingested by monarchs in the field are expected to be lower than levels fed to them in the Hellmich *et al.* laboratory study.

The Karner blue feeds and oviposits on wild lupines (*Lupinus perennis*). They are found in parts of Wisconsin, Minnesota, Indiana, New Hampshire, and New York. Wild lupines are typically found in pine barrens, oak savanahs, forest trails, and other previously disturbed habitats. Wild lupines are not expected to occur in corn fields or within a few meters of corn fields. Karner blues first generation hatches in mid-April and adults emerge in late May to early June. Second generation Karner blues hatch in late may and feed through mid July. Since corn typically pollinates in late July to early August, or some time after mid-July, they are not expected to be feeding when corn pollen is abundant.

It is unacceptable to test the Karner blue butterfly (*Lyceides melissa*) because it is an endangered species. The ABSTC suggests that testing a close relative of the Karner blue would not necessarily provide an accurate assessment of the Karner blue's susceptibility to Cry1Ab. Previous studies that tested the susceptibility of lepidopterans to Cry1Ab resulted in different LC_{50} values for different species in the same genus. For example the Cry1Ab LC_{50} for *Spodoptera exigua* is estimated at 3180 ng/mL diet and 95890 ng/mL diet for *Spodoptera frugiperda* (Luttrell *et al.* 1999). The ABSTC also suggested that the Karner blue's susceptibility to Cry1Ab is similar to the European corn borer and monarch larvae. Since levels of Bt pollen found in the field are not toxic to European corn borers, levels toxic to the Karner blue are not expected. One study demonstrated that the Karner blue has similar susceptibility to a microbial Bt containing Cry1 proteins as the gypsy moth (Herms *et al.* 1997). Since the gypsy moth is known to be less susceptible to Bt than European corn borers, the Karner blue is also expected to have a lower level of susceptibility.

<u>BPPD Conclusions</u>:

A laboratory study showed that the LD_{50}s for purified Cry1Ab protein fed to ECB larvae in artificial diet are approximately 3.29 ng/mL diet for first instars; 35.1 ng/mL diet for second to third instars; and >100 for third and fourth instars (Hellmich *et al.* 2001). Since monarchs are probably exposed to varied amounts of Bt in the field and the activity of Bt in pollen declines over time, the LD_{50}s determined from this laboratory experiment are probably higher than exposure in the field.

Since susceptibility of the Karner blue is not necessarily equivalent to other species from the genus Lycaeides and tests cannot be conducted with the Karner blue, determining an LC_{50} is difficult to impossible. Herms *et al.* (2001) demonstrated that Karner blue butterflies are susceptible to Cry1Ab. Therefore, it can be assumed that the Karner blue is susceptible to MON810 and Bt11 and will be adversely affected if toxic levels of pollen are ingested. However, the Karner blue is probably no more sensitive to Cry1Ab than monarch butterflies and will not consume toxic levels of Bt in the field. In addition, Karner blue habitat which includes wild lupines in pine barrens and oak savannahs do not typically occur near corn fields. Therefore, Karner blue butterflies are probably not adversely affected by MON 810 and Bt11 in the field. However, wild lupines may occur near some corn fields in areas such as Wisconsin where the weed grows in fallow fields and promotes Karner blue establishment. If Bt corn is planted in these fields where wild lupines have been established in following years, exposure to MON 810 and Bt11 may occur. Therefore, the Agency is initiating an informal consultation with the Fish and Wildlife Service (FWS) to determine potential impacts of Bt corn on Karner blue butterflies.

DCI Data Element 7:

(7) Determine the monarch larvae LD50's for pollen, for representative inbreds and hybrids from your transformation event(s), containing your Bt corn plant-pesticide and report the results on both a weight and number of pollen grains basis.

Classification: Acceptable

Summary of ABSTC's response:

Bt proteins are typically expressed in low to unquantifiable levels in corn pollen. Levels of Bt proteins in corn pollen were reported as: 1. MON 810 (Cry1Ab) = <90 ng/g dry wt. pollen; 2. Bt11 (Cry1Ab) = <90 ng/g dry wt. pollen; 3. Event 176 (Cry1Ab) = 5000 ng/g dry wt. pollen; 4. CBH351 (Cry9C) = 240 ng/g dry wt. pollen. Cry 1Ab is only found in pollen of the currently registered Bt field corn hybrids (MON 810 and Bt11) in trace quantities (<90 ng/g dry wt. pollen). Event 176 (Cry1Ab) has the highest level of Bt protein expressed in pollen because of a pollen-specific promoter. However, only 2% of the U.S. corn acreage in 2000 consisted of Event 176 hybrids and there will be less acreage planted as this product is phased out.

Inbred corn lines which are homozygous for the *Bt* gene are expected to express twice the level of Bt as hybrids which are hemizygous for the *Bt* gene. Since inbreds are only found in small, isolated fields used for seed production that are kept clean of weeds and occur on less than 1% of the corn acreage, monarch exposure to inbred corn pollen is expected to be minimal. Therefore, pollen expressing Bt from inbred lines do not present a greater risk to monarchs that hybrid corn pollen. In addition, Bt popcorn occurs on <0.5% and Bt sweet corn occurs on <1% of acreage corn acreage. Therefore, Bt popcorn and Bt sweet corn pollen are not expected to pose a risk to monarchs because exposure is insignificant and similar to exposure to inbred lines.

A four day laboratory study exposing first instars to milkweed leaves naturally dusted with pollen in the field resulted in no observable effects of MON 810 and Bt11 on survival and fitness of monarchs. The highest level of pollen averaged 504-586 grains/cm^2 and occurred on milkweeds located 3 m inside a Bt11 sweet corn in Maryland (Stanley-Horne *et al.* 2001). In Ontario, monarchs were fed milkweeds from within corn fields that had an average of 59 - 97 grains/cm^2 Bt11 field corn pollen for five days and then reared to adults. A field study in Ontario showed no statistically significant differences between monarchs feeding on Bt11 pollen and non-Bt pollen on survival, leaf consumption (cm^2), developmental change, weight gain, percent survival to pupation, pupal weight (mg), percent emergence from pupae, adult weight (mg), or adult wing length (Stanley-Horne *et al.* 2001).

A study conducted by Hellmich *et al.* (2001) involved feeding first instar monarchs no pollen or known amounts of Bt (MON 810 and Bt11) and non-Bt pollen applied in the laboratory. Since extremely high pollen levels were used and controlled conditions were used so no pollen was removed due to environmental factors, these condition are considered a worst case scenario. Pollen levels ranged from 250 - 2000 grains/cm^2 for MON 810 and 150 - 4000 grains/cm^2 for Bt11 and resulted in no significant effects on larval weight. In a laboratory study, black swallowtails were fed densities of up to 10,000 grains/cm^2 of MON810 and no effects on larval fitness were observed (Wraight *et al.* 2000). Since larval fitness (e.g., weight) is a very sensitive indicator of potential adverse effects, yield effects on survival and fitness are not expected. In fact, sensitivity is expected to decrease with older larval stages.

Jesse and Obrycki (2000) reported larval weight gain effects of Bt11 at 135 grains/cm^2 and Head and Brown (1999) showed mortality at 4000 grains/cm^2 of MON 810. However, these studies should be considered with caution since they did not thoroughly filter additional debris (e.g., anthers) from the pollen that would probably not accumulate on milkweed leaves in the field to a significant degree (Hellmich *et al.* 2001, Pleasants *et al.* 2001). Hellmich *et al.* (2001) showed that larvae feeding on pollen containing other particles gained significantly less weight than larvae feeding on finely filtered pollen with no debris.

<u>BPPD Conclusions</u>:

Cry 1Ab is only found in pollen of the currently registered Bt field corn hybrids (MON 810 and Bt11) in trace quantities (<90 ng/g dry wt. pollen). First instar monarch larvae is the most sensitive stage Bt. First instar larvae feeding on milkweed leaves naturally dusted with pollen in the field resulted in no observable effects of MON 810 and Bt11 on survival and fitness of monarchs. The highest level of pollen averaged 504-586 grains/cm^2 and occurred on milkweeds located 3 m inside a Bt11 sweet corn in Maryland (Stanley-Horne *et al.* 2001). Extremely high pollen levels (250 - 2000 grains/cm^2 for MON 810 and 150 - 4000 grains/cm^2 for Bt11) were fed to first instar monarch larvae in a controlled environment and resulted in no significant effects on larval weight (Hellmich *et al.* 2001). From this data, it can be concluded that the NOEL for MON 810 is >2000 grains/cm^2 an for Bt11 is >4000 grains/cm^2 which is greater than levels that occur under natural field conditions.

DCI Data Element 8:

(8) Determine and report each instar larval survival and developmental effects in the presence of Bt pollen, for representative inbreds and hybrids from your transformation event(s), containing your Bt corn plant-pesticide.

Classification: Acceptable

Summary of the ABSTC's response:

In general, first instar lepidopteran are more sensitive to Bt than older larvae (Gould 1994). Based on this assumption, the1998 Scientific Advisory Panel (SAP) Subpanel on Bt Plant-Pesticides and Resistance Management recommended testing later instars to establish a high-dose (US EPA 1998). In a study conducted by Hellmich *et al.* (2001), monarch larvae were fed purified trypsin resistant core of various Bt Cry proteins (Cry1Ab, Cry1Ac, Cry9C, and Cry1F) incorporated in diet to determine LC_{50} and EC_{50} values in ng/mL diet.

Instar	LC_{50}	EC_{50}
1st	3.29	0.76
2nd - 3rd	35.1	9.60
3rd - 4th	>100	18.3

Results of the Hellmich *et al.* test used purified Cry1Ab; therefore, the LC_{50}s and EC_{50}s reported probably overestimate potential toxicity of Bt pollen to monarch larvae. This study showed that older monarch larvae are more susceptible to Cry1Ab protein than younger larvae.

No observable effects from feeding on Bt corn pollen expressing MON 810 and Bt11 on survival, weight or other fitness parameters of early and late instar monarch larvae were found by Stanley-Horne *et al.* (2001). This data demonstrated that older monarch larvae are less sensitive to MON 810 and Bt11 pollen than early instars. See answer to DCI 7 for details of this study.

Inbred corn lines which are homozygous for the *Bt* gene are expected to express twice the level of Bt as hybrids which are hemizygous for the *Bt* gene. Since inbreds are only found in small, isolated fields used for seed production that are kept clean of weeds and occur on less than 1% of the corn acreage, monarch exposure to inbred corn pollen is expected to be minimal. Therefore, pollen expressing Bt from inbred lines do not present a greater risk to monarchs that hybrid corn pollen. In addition, Bt popcorn occurs on <0.5% and Bt sweet corn occurs on <1% of acreage corn acreage. Therefore, Bt popcorn and Bt sweet corn pollen are not expected to pose a risk to monarchs because exposure is insignificant and similar to exposure to inbred lines.

BPPD's Conclusions:

Studies conducted since this DCI was initiated have shown that monarch larvae feeding on corn pollen expressing MON 810 or Bt11 do not demonstrate observable adverse effect on survival, weight, or other fitness parameters (e.g., developmental change, weight gain, percent survival to pupation, pupal weight (mg), percent emergence from pupae, adult weight (mg), or adult wing length). These studies have also shown that young monarch larvae are more sensitive to Bt than older larvae (Hellmich *et al.* 2001, Stanley-Horne *et al.* 2001). The LC_{50}s for the various monarch larval stages were found to be: 1. 1^{st} instars = 3.29 ng Cry1Ab/mL treated artificial diet; 2. 2^{nd} - 3^{rd} instars = 35.1 ng Cry1Ab/mL treated artificial diet; 3. 3^{rd} - 4^{th} instars = >100 ng Cry1Ab/mL treated artificial diet (Hellmich *et al.* 2001).

DCI Data Element 9:

(9) The Cornell data (Losey, J., L. Raynor, and M. Carter. 1999. Transgenic pollen harms monarch larvae. Nature 399:214.) show that less larval feeding took place on pollinated milkweed leaves than on non-pollinated leaves. Therefore:

9(a) Determine and report what is the probability of pollen consumption by monarch larvae on encounter on Milkweed leaves.

9(b) Determine and report whether foraging larvae actively avoid Bt-pollen, for representative inbreds and hybrids from your transformation event(s); in the field;

9(c) Determine and report whether monarch larvae avoid feeding on non-Bt corn pollen under field conditions;

9(d) Do monarchs avoid feeding on Bt corn pollen, for representative inbreds and hybrids from your transformation event(s), under field conditions?

9(e) Determine and report whether monarchs are avoiding corn fields for preferred areas to feed.

Classification: Acceptable

<u>Summary of ABSTC's response</u> :

In laboratory bioassays, Hellmich *et al.* (2001) found that monarchs prefer to feed on milkweed leaves dusted with a moderate amount of Bt and non-Bt pollen (e.g., ~60 pollen grains/cm^2) than leaves with no pollen. However, monarch larvae bioassayed in this study preferred milkweed leaves with no pollen over leaves dusted with high amounts of Bt or non-Bt pollen (~600 pollen grains/cm^2) with one exception for MON 810 (Hellmich *et al.* 2001). Since expression of MON 810 and Bt11 in corn pollen are similar, observations of feeding on MON 810 should be similar to Bt11. The Hellmich *et al.* (2001) results are consistent with Losey *et al.* (1999) which showed that monarch larvae prefer to feed on non-Bt than Bt pollen. Another Losey *et al.* (2000) study have showed that monarchs either do not show a preference for milkweeds with or without corn pollen dusted on them, or they avoid pollen dusted milkweeds (Losey *et al.* 2000).

In addition, field studies showed that monarchs will oviposit on milkweeds growing in Bt and non-Bt corn fields (Oberhauser *et al.* 2001). Therefore, the ABSTC concluded that female monarchs either do not avoid ovipositing in corn fields or prefer to oviposit in corn fields. However, they may avoid ovipositing on milkweeds covered with high pollen densities.

Distribution of milkweed leaves in five habitats was determined by monitoring in representative geographic areas monarchs are expected to breed including east-central Minnesota, west-central Wisconsin, central Iowa, coastal Maryland, and southern Ontario. Milkweed plants were monitored for egg and larval populations in a corn field (all non-transgenic except for one transgenic corn field in Maryland), near corn field edges, in non-agricultural areas (e.g., old field, restored prairie, or other non-crop habitat), or other non-agricultural crops (Iowa and Maryland sites only) pre- and post-anthesis (Oberhauser *et al.* 2001).

The Oberhauser *et al.* (2001) field studies showed that monarch larvae will be exposed to corn pollen in the field and supported results from the Hellmich *et al.* (2001) and Losey *et al.* (2001) laboratory studies which showed that monarch larvae will feed on milkweed plants dusted with corn pollen. However, monarchs feeding on the moderate levels of MON 810 and Bt11 expressing pollen that larvae are typically exposed to in the field are not adversely affected

Inbred corn lines which are homozygous for the *Bt* gene are expected to express twice the level of Bt as hybrids which are hemizygous for the *Bt* gene. Since inbreds are only found in small, isolated fields used for seed production that are kept clean of weeds and occur on less than 1% of the corn acreage, monarch exposure to inbred corn pollen is expected to be minimal. Therefore, pollen expressing Bt from inbred lines do not present a greater risk to monarchs that hybrid corn pollen. In addition, Bt popcorn occurs on <0.5% and Bt sweet corn occurs on <1% of acreage corn acreage. Therefore, Bt popcorn and Bt sweet corn pollen are not expected to pose a risk to monarchs because exposure is insignificant and similar to exposure to inbred lines.

BPPD Conclusions:

Since monarch eggs and larvae were found on milkweed plants in the field when pollen was present on leaves (Oberhauser *et al.* 2001), there is a high probability that monarchs will encounter Bt corn pollen. In addition, it can be assumed that monarch larvae will consume both Bt or non-Bt pollen if it is encountered (Hellmich *et al.* 2001, Oberhauser *et al.* 2001). Laboratory (Hellmich *et al.* 2001, Losey *et al.* 2001) and field studies (Oberhauser *et al.* 2001) demonstrated that monarchs will not avoid feeding on plants dusted with Bt or non-Bt corn pollen. Surveys conducted in Minnesota, Wisconsin, and Iowa suggest that monarchs will oviposit in corn fields 45 to 107 times more often than in nonagricultural habitats (Oberhauser *et al.* 2001). Since eggs and larvae were found on milkweed plants naturally dusted in corn pollen in the field, it can be assumed that foraging monarchs will not avoid pollen dusted plants nor do they avoid corn fields.

DCI Data Element 10:

(10) Determine and report whether there are practical ways of decreasing the potential of monarchs encountering or feeding upon Bt pollen.

Classification: Acceptable

Summary of ABSTC's response:

The ABSTC has requested a waiver for DCI data element 10 because of the lack of toxicity from the pollen of MON 810 and Bt11.

BPPD Conclusions:

From the surveys conducted in response to this DCI, the highest level of pollen found in the field averaged 504-586 grains/cm^2 and occurred on milkweeds located 3 m inside Bt11 sweet corn in Maryland (Stanley-Horne *et al.* 2001). Extremely high pollen levels (250 - 2000 grains/cm^2 for MON 810 and 150 - 4000 grains/cm^2 for Bt11) were fed to first instar monarch larvae in a controlled environment and resulted in no significant effects on larval weight (Hellmich *et al.* 2001). Therefore, it can be concluded that levels of MON 810 or Bt11 pollen toxic to monarch larvae do not occur under natural field conditions.

DCI Data Element 11:

(11) Determine and report whether monarchs have a site preference for egg laying

11(a) Determine and report whether monarch adults oviposit on or avoid milkweeds near corn fields.

Classification: Acceptable

Summary of ABSTC's response:

Studies have shown that monarchs either do not show a preference for milkweeds with or without corn pollen dusted on them, or they avoid pollen dusted milkweeds (Losey *et al.* 2000). Monarchs will oviposit on milkweeds growing in Bt and non-Bt cornfields (Oberhauser *et al.* 2001). In surveys conducted in Maryland and Wisconsin, monarchs did not show an ovipositional preference for milkweeds found in corn fields, near field edges, other agricultural fields, or non-agricultural areas. However, in surveys conducted in Minnesota and Wisconsin, monarchs preferred to oviposit on milkweeds found in corn fields than non-agricultural habitats and were not affected when Bt or non-Bt pollen was present (Oberhauser *et al.* 2001). Although all of the surveys showed milkweeds were shorter in corn fields than non-agricultural areas, new attractive growth occurred in the upper midwest due to plowing, mid-season cultivation, and/or fertilizing (Oberhauser *et al.* 2001).

Therefore, the ABSTC concluded that female monarchs either do not avoid ovipositing in corn fields or prefer to oviposit in corn fields. However, they may avoid ovipositing on milkweeds covered with high pollen densities.

BPPD Conclusions:

Results vary regarding monarch preference, avoidance, or indifference to ovipositing on milkweeds in corn fields (Losey *et al.* 2000, Oberhauser *et al.* 2001). Surveys conducted by Oberhauser *et al.* found monarch eggs on milkweeds dusted with pollen in and near corn fields. Therefore, it can be assumed that monarchs do not avoid ovipositing on milkweeds in or near corn fields. In some instances monarchs may prefer to oviposit on milkweeds occurring within corn fields (Oberhauser *et al.* 2001). Otherwise, surveys showed that monarchs do not appear to have an ovipositional preference for milkweeds found in corn fields, near field edges, other agricultural fields, or non-agricultural areas (Oberhauser *et al.* 2001).

DCI Data Element 12:

(12) Determine and report what is the effect of Bt corn pollen presence, for representative inbreds and hybrids from your transformation event(s), on monarch oviposition behavior

12(a) Determine and report whether monarchs deposit eggs on non-Bt corn pollinated milkweed under field conditions.

12(b) Determine and report whether monarchs adults deposit eggs on Bt corn pollinated milkweed under field conditions.

Classification: Acceptable

Summary of ABSTC's response:

Studies have shown that monarchs either do not show a preference for milkweeds with or without corn pollen dusted on them, or they avoid pollen dusted milkweeds (Losey *et al.* 2000). Monarchs will oviposit on milkweeds growing in Bt and non-Bt cornfields (Oberhauser *et al.* 2001). In surveys conducted in Maryland and Wisconsin, monarchs did not show an ovipositional preference for milkweeds found in corn fields, near field edges, other agricultural fields, or non-agricultural areas. However, in surveys conducted in Minnesota and Wisconsin, monarchs preferred to oviposit on milkweeds found in corn fields than non-agricultural habitats and were not affected when Bt or non-Bt pollen was present (Oberhauser *et al.* 2001). Although all of the surveys showed milkweeds were shorter in corn fields than non-agricultural areas, new attractive growth occurred in the upper midwest due to plowing, mid-season cultivation, and/or fertilizing (Oberhauser *et al.* 2001).

Therefore, the ABSTC concluded that female monarchs either do not avoid ovipositing in corn fields or prefer to oviposit in corn fields. However, they may avoid ovipositing on milkweeds covered with high pollen densities.

BPPD Conclusions:

Results vary regarding monarch preference, avoidance, or indifference to ovipositing on milkweeds in corn fields (Losey *et al.* 2000, Oberhauser *et al.* 2001). Surveys conducted by Oberhauser *et al.* found monarch eggs on milkweeds dusted with pollen in and near corn fields. Therefore, it can be assumed that monarchs do not avoid ovipositing on milkweeds in or near corn fields. In some instances monarchs may prefer to oviposit on milkweeds occurring within corn fields (Oberhauser *et al.* 2001). Otherwise, surveys showed that monarchs do not appear to have an ovipositional preference for milkweeds found in corn fields, near field edges, other agricultural fields, or non-agricultural areas (Oberhauser *et al.* 2001).

12(c) Determine and report whether monarch adults oviposit on milkweeds (under leaves, in inflorescents).

Summary of ABSTC's response:

Borkin (1982) surveyed milkweed plants in southeastern Wisconsin and found that monarch eggs were typically layed singly, but up to three eggs were found per leaf. Most monarch eggs are layed on the underside of leaves (Urquhart 1960, Borkin 1982) and some eggs were found on the dorsal surface, stalks or flowers (Borkin 1982). In general, females prefer to lay eggs on young tender plants and tissue (Urquhart 1960, Borkin 1982). Losey *et al.* (2001) also showed that monarchs prefer to oviposit on the underside (95% of plants surveyed) of the topmost leaves (76% of plants surveyed) on the milkweed plant which represent the youngest tissue. Pollen has been shown to adhere to the underside of milkweed leaves in insignificant amounts in the field (Oberhauser *et al.*2001, Pleasants *et al.* 2001). Monarch ovipositional preference did not appear to be influenced by the presence of pollen in the field (Pleasants *et al.* 2001).

BPPD Conclusions:

Surveys have shown that monarchs prefer to oviposit single eggs on the underside of milkweed leaves on young, tender tissue (Urquhart 1960, Borkin 1982, Pleasants *et al.* 2001). However, females may lay more than one egg per plant and may oviposit on the top of leaves, on stalks or flowers (Borkin 1982). Studies conducted by Oberhauser *et al.* (2001) and Pleasants *et al.* (2001) showed that monarchs will oviposit and feed on milkweed leaves dusted with pollen in the field.

DCI Data Element 13:

(13) Confirm and report where the various instars of monarch larvae feed on the milkweed plant:

13(a) upper vs. lower leaves

13(b) also determine and report on feeding behavior regarding upper and undersides of leaves (changes exposure considerably)

13(c) shoot apex vs tops of leaves

Classification: Acceptable

Summary of the ABSTC's response:

Borkin (1982) surveyed milkweed plants in southeastern Wisconsin and found that monarch eggs were typically layed singly, but up to three eggs were found per leaf. Most monarch eggs are layed on the underside of leaves (Urquhart 1960, Borkin 1982) and some eggs were found on the dorsal surface, stalks or flowers (Borkin 1982). In general, females prefer to lay eggs on young tender plants and tissue (Urquhart 1960, Borkin 1982). Losey *et al.* (2001) also showed that monarchs prefer to oviposit on the underside (95% of plants surveyed) of the topmost leaves (76% of plants surveyed) on the milkweed plant which represent the youngest tissue. First and second instar larvae tend to feed on younger plant tissue; whereas, older larvae appear to be less discriminating (Urquhart 1960).

Monarch larvae also prefer to feed on young tissue and first instars are typically found feeding on the underside of leaves because their mandibles are not developed enough to penetrate tougher tissue (e.g., tops of leaves). However, sometimes first instar larvae will feed on the tops of leaves but they do not feed on leaf veins or veinlets. As larvae age, their mandibles develop and become stronger allowing them to feed through leaf tissue, or feed on the tops of leaves and on veins (Urquhart 1960). Second instar larvae have also been reported to feed on milkweed leaves close to where eggs are layed. Since eggs are typically layed on the underside of milkweed leaves, neonate larvae are usually found feeding on the underside of leaves. However, age of plant tissue appears to have a greater influence on larval feeding (Urquhart 1960). Urquhart (1960) also found that larvae may move between food plants during development.

BPPD Conclusions:

Surveys have shown that monarchs prefer to oviposit single eggs on the underside of milkweed leaves on young, tender tissue (Urquhart 1960, Borkin 1982, Pleasants *et al.* 2001). However, females may lay more than one egg per plant and may oviposit on the top of leaves, on stalks or flowers (Borkin 1982). Age of plant tissue is probably the most important influence on monarch ovipositional preference. Female monarchs prefer to oviposit on young tender plat tissue (Urquhart 1960, Borkn 1982). Neonate larvae begin feeding near the area eggs were layed which is typically under leaves. As larvae mature, they may feed through leaves, on top of leaves, or on leaf veins (Urquhart 1960).

DCI Data Element 14:

(14) Determine and report how long the lethal concentration of Bt corn pollen, for representative inbreds and hybrids from your transformation event(s), stays on milkweed leaves.

14(a) Determine and report how long Bt in corn pollen retains its toxicity.

14(b) Determine and report whether sunlight degrades Bt toxin in corn pollen on milkweed, and

14(c) Determine and report whether wind, rain or other environmental factors remove Bt pollen from milkweed.

Classification: Acceptable

Summary of ABSTC's response:

Studies have shown that levels of Bt corn pollen that occur in the field are not toxic to monarch butterfly larvae (Hellmich *et al.* 2001, Oberhauser *et al.* 2001, Stanley-Horne *et al.* 2001). Bt proteins are also known to breakdown rapidly when exposed to ultraviolet light, heat or biological activity (Gelernter 1990). Bt's rapid breakdown and the fact that corn pollen loses it's viability a few hours after release from the plant suggests that Bt insecticidal activity in pollen should degrade rapidly. Insect bioassays with pollen expressing MON 810 that was exposed to natural light showed detectable bioactivity was lost within seven days (Head and Brown 1999). Since Bt11 pollen also has only trace levels of Cry1Ab protein, data collected on MON 810 can be bridged to Bt11.

Rain, wind, gravity, and plant architecture are known to remove pollen from milkweed leaves. However, rainfall has the greatest impact on removing pollen from milkweed leaves (Pleasants *et al.* 2001). Pleasants *et al.* (2001) found that 86% of the pollen on milkweed leaves was removed by a single rainfall. In Ontario, 92% of pollen collected on sticky slides in the field was removed after several rainfalls (Pleasants *et al.* 2001). Stanley-Horne *et al.* (2001) also found rainfall to significantly reduce pollen on milkweed leaves. Irrigation will also remove pollen from milkweed leaves. In Nebraska, for example, approximately 5.6 million acres of corn is irrigated with the highest water use occurring during pollination (Benham 1998).

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BPPD Conclusions:

Microbial enzymes, secondary plant compounds, extremes in pH, ultraviolet light, wind and rain degrade Bt proteins in microbial sprays. The insecticidal activity in Bt microbial sprays has been shown to break down rapidly for two days after application and is practically nonexistent four days post application (Gelernter 1990). Head and Brown (1999) only found biological activity of MON 810 in fresh pollen. Laboratory assays showed that MON 810 activity was not detectable in pollen after seven days (Head and Brown 1999). The biological activity of Bt proteins probably decreases more rapidly in the field where it is exposed to elements such as ultraviolet light than in the laboratory. Therefore, the Bt may breakdown more rapidly than seven days under field conditions. In addition, rainfall has been shown to remove most (86 - 92%) of the pollen from milkweed leaves, thus potentially reducing the length of monarch exposure to Bt pollen (Pleasants *et al.* 2001, Stanley-Horne *et al.* 2001).

DCI Data Element 15:

(15) Determine and report how soon after planting do representative inbreds and hybrids from your transformation event(s) pollinate.

Classification: Acceptable

Summary of the ABSTC's response:

Corn hybrids do not pollinate according to calendar date; rather, they pollinate according to Growing Degree Unites (GDU). The GDU units needed for pollination to occur varies among hybrids and is publicly available in seed company catalogs. Hybrids suited for different regions will typically pollinate at different times for example a hybrid targeted for Minnesota would probably pollinate sooner than one grown in Southern Iowa. Bt corn hybrids have been developed for most corn growing regions and vary from 1000 GDU for short season hybrids (Northern) to 1500 GDU for full season hybrids (Southern) until pollination. Examples of the approximate number of GDU needed for pollination to occur in different regions are:

Area of Adaptation	~GDU to pollination for a representative adapted hybrid
Fargo, ND	1130
Madison, WI	1250
Lincoln, NE	1370
Champaign, IL	1390
Salisbury, MD	1400
Lubbock, TX	1450

Although GDUs to pollination for inbreds is not published in seed catalogs because these they are not sold commercially, it can be assumed that time to pollination is similar for inbreds and commercial hybrids.

BPPD Conclusions:

Time to pollination varies among hybrids and regions and is determined according to GDUs. GDUs to pollination can be found in commercial seed catalogues. Examples of the approximate number of GDU needed for pollination to occur in different regions are:

Area of Adaptation	~GDU to pollination for a representative adapted hybrid
Fargo, ND	1130
Madison, WI	1250
Lincoln, NE	1370
Champaign, IL	1390
Salisbury, MD	1400
Lubbock, TX	1450

DCI Data Element 16:

(16) Determine and report whether the duration of pollination for each *corn ear* and the *total field* match the expected 3 and 13 days, respectively, for representative inbreds and hybrids from your transformation event(s).

Classification: Acceptable

Summary of the ABSTC's response:

Weather has a greater effect on the length of time silks are receptive to pollination than genetics. All silks on a corn ear are typically exposed to pollination within two to three days (Ritchie *et al.* 1997). However, silks and/or pollen grains may dessicate in the field leading to a reduction in the time corn ears can be pollinated.

A single tassel will typically shed pollen for two days to longer than a week (Russell and Hallauer 1980). Variability in field conditions may result in an increase in the duration of pollen-shed. A study conducted by Bassetti and Westgate (1994) showed pollen-shed in one field in Minnesota occurred over a 15-day period with approximately four days of pollen-shed per plant. Inbreds generally have a shorter pollination period (Russell and Hallauer 1980) which is reduced by adverse growing conditions.

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BPPD Conclusions:

Individual corn tassels typically shed pollen for two to seven days (or longer) and silks on an ear are exposed to pollination for two to three days (Russell and Hallauer 1980, Ritchie *et al.* 1997). A field will shed pollen for up to 15 days depending upon microclimate (Russell and Hallauer 1980).

Although the response submitted by the ABSTC does not directly address the Bt corn crops, the response is sufficient for the Agency's purposes at this time.

DCI Data Element 17:

(17) Determine and report whether the monarch larvae are feeding on milkweeds during pollen shed.

(a) If so, determine and report how long is the regional overlap of time when the monarch larvae are exposed to corn pollen.

(b) Determine and report what fraction of monarch larvae could be exposed to corn pollen, considering that in any specific region the corn is shedding pollen for only a week to ten days each year.

(c) Determine and report the probability of monarch larvae encountering pollen from Bt corn.

Classification: Acceptable

Summary of the ABSTC's response:

To describe the probability of the emergence and feeding of monarch larvae spatially and temporally concurrent with pollen shed within corn growing regions, model simulations were run and Regional maps were produced by Calvin *et al.* (2000). The time populations of the various monarch larval stages appeared were overlain on regional maps with pollen release. Emergence of various instar populations was predicted by modeling timing of corn pollination and monarch phenology. Arrival times of first and second generation monarchs were coupled with growing-degree-day information at a location. At a given latitude, pollen shed is normally distributed for the earliest and latest maturing hybrids between predicted dates of pollination (Calvin *et al.* 2000). Model simulations for monarch and corn development were developed for seven states along the central plains and one state at the northeastern migratory route (Rock Spring, PA; 40.5° north latitude). States along the central plains occurred along 1-degree north latitude increments and included: Lawrence, KS (39° north), Champaign, IL (40° north), Mason City, IA (43° north), Rochester, MN (44° north), and St. Paul, MN (45° north).

At the Lawrence Kansas location, the model predicted that there is minimal overlap of early instar monarch larvae and corn pollen shed. Although the degree of early instar occurrence and pollination overlap varied among locations, these models showed that the degree of co-occurrence generally increased as latitude increased. A partial degree of second and third instar overlap with pollination, which was the least degree of synchrony, was predicted in Lincoln, NE. The degree of overlap increased from Ames, IA northward to St. Paul, MN. Examples of areas that the model predicted almost 100% overlap of corn pollination with the occurrence of early instars were Mason City, IA, Rochester, MN, St. Paul, MN and Rock Spring, PA (ca. 40.5 degrees north latitude). As latitude increased, there was a higher degree of overlap between occurrence of monarch larvae and corn pollen shed that was probably the result of cooler temperatures at increased elevation (Calvin 2000).

These models also showed a general increase in the level of second generation early instar monarch overlap with corn pollination from 39 to 45 degrees north latitude. The overlap between early instar monarchs and corn pollination approaches 100% between 42 and 43 degrees north latitude. There is more overlap at higher elevations occurring at lower latitudes. The highest level overlap occurs at the northern extremities of the corn belt. Monarch overlap with corn pollination appeared to be negligible within most of the Corn Belt.

It was also concluded that spring arrival date does not effect the level of early instars and corn pollination overlap to any significant degree. In addition, early spring arrival can influence the length of each life stage. In general, Calvin et al. (2000) models show that developmental cycles of monarchs may result in a lack of co-occurrence of early monarch instar populations with pollen shed throughout most of the Corn Belt. There may be more at the northern extreme of the Corn Belt as well as with higher elevation in the northeastern U.S where less corn is grown.

Additional model simulations that utilized 2000 temperature data from each location had varied results for Maryland, Iowa, Minnesota, Wisconsin, and Ontario. In general, model predictions for the degree of overlap of monarch larvae and pollen shed were close to field predictions in northern locations except for Ames, IA where most of the corn was planted early in 2000. Area-wide pollination varied between three to four weeks among locations and varied seven to 14 days for a single field. These results suggest that not all monarch larvae will be exposed to corn pollen during the area-wide pollination period even where 100% overlap is expected (Calvin *et al.* 2000).

Surveys conducted by Oberhauser *et al.* 2001 generally agreed with the predictions of the Calvin *et al.* (2000) models. Four different monarch breeding regions (east-central Minnesota and west-central Wisconsin, central Iowa, coastal Maryland, and southern Ontario) were monitored when monarchs were present. Results showed temporal overlap of monarch larvae and corn pollination were:

State	% Overlap of Larvae & Anthesis	% Overlap of Migratory Gen. Larvae and Anthesis
Minnesota	20% to 68%	50%
Ontario	27% to 75%	50%

State	% Overlap of Larvae & Anthesis	% Overlap of Migratory Gen. Larvae and Anthesis
Maryland	0 to 36%	15%
Iowa	4% to 25%	15%
Wisconsin		50%

It can be concluded from the models (Calvin *et al.* 2001) and field surveys (Oberhauser *et al.* 2001) that monarch larval occurrence and corn pollen shed overlap is negligible in southern and central parts of the Corn Belt and there is up to 75% overlap in the northernmost area of the Corn Belt. This means that 0 to 5% of monarchs will be exposed to Bt corn pollen in most of the Corn Belt and 10% exposure will occur in the northern region.

BPPD Conclusions:

Oberhauser *et al.* (2001) and Pleasants *et al.* (2001) showed that monarchs do occur on milkweeds in the field during pollen shed. Therefore, monarch will potentially encounter Bt pollen in the field. According to models developed by Calvin *et al.* (2000) and Oberhauser *et al.* (2001), overlap of monarch larval occurrence and corn pollination is negligible in southern and central parts of the Corn Belt, but there is up to 75% overlap in the northernmost area of the Corn Belt. This means that 0 to 5% of monarchs will be exposed to Bt corn pollen in most of the Corn Belt and 10% exposure will occur in the northern region. In general, the Calvin *et al.* (2000) model showed that the degree of co-occurrence generally increased as latitude or elevation increased.

DCI Data Element 18:

(18) Determine and report whether monarchs carrying pollen on their exoskeleton and distribute it on milkweeds during egg deposition. If so, determine the quantity.

Classification: Acceptable

Summary of ABSTC's response:

Monarchs must ingest Bt corn pollen for toxicity to occur. Therefore, the ABSTC claims that monarchs are not at risk if exposed to Bt corn pollen through the exoskeleton and exposure of adults to Bt pollen is minimal. Pollen grains would have to adhere to female monarchs and fall onto and adhere to milkweed plants during oviposition for exposure to potentially occur. Any pollen that may adhere to adults would probably dislodge due to wind currents or during oviposition. Since monarchs typically lay eggs singly on the underside of milkweed leaves, pollen would fall away from eggs. There is also no danger of pollen landing on larvae since there is only one egg laid per plant. The ABSTC believes that the amount of pollen that could be carried on a monarch exoskeleton is inconsequential.

BPPD Conclusions:

The level of exposure of monarch larvae to Bt pollen carried to milkweed plants on exoskeleton of adults is minimal. If pollen were to adhere to monarch adults and dislodged on milkweeds, quantities would not be great enough to adversely affect larva feeding on these milkweeds. Since Bt must be ingested and will not harm monarchs by contacting it's exoskeleton, there is minimal to risk posed from monarchs transporting pollen among milkweed plants.

DCI Data Element 19:

(19) Determine and report what is the risk of monarch exposure to Bt corn pollen in the context of other significant risk factors impacting monarch survival and population size (e.g. conventional and microbial insecticides, herbicides, destruction of overwintering sites, predation, cars, etc).

Classification: Acceptable

Summary of ABSTC's response:

The ABSTC has requested that this data requirement be waived since they have concluded that Bt corn pollen poses minimal risk to monarchs. Many risks to overwintering and migrating monarchs have previously been identified. Predators, particularly fire ants and spiders, of migrating monarchs are a major risk to monarchs. Impacts to monarchs also occur due to weather, physiological stress (mainly starvation), and a small anthropogenic component (human activities such as rural to urban/suburban land use patterns and vehicle impact) (Taylor 1999). Destruction of overwintering sites, cropping practices, herbicide and insecticide use may also cause risks to monarchs.

From 1986 - 1993, approximately 11% of the corn acreage in the northern and southern plain states were treated with insecticides for ECB control (Martiz Marketing Research 1987-1994 summarized in Hamblin *et al* 1994). Typically, contact broad-spectrum organophosphates, carbamates and synthetic pyrethroids are sprayed. According to a grower survey conducted by Rice and Pilcher (1999), 26% of corn growers reduced their insecticide treatments for ECB due to planting Bt corn. Therefore, Bt corn is likely reducing insecticide use and potential risk to ECB and other non-target insects .

Potential adverse effects of insecticide use is most apparent in Bt sweet corn. Although only 1% of corn acreage in the U.S. is planted with sweet corn, approximately 40% of all corn insecticide applications for lepidopteran control occur in these fields. Stanley-Horne *et al.* (2001) showed no significant mortality or weight gain difference between first instar larvae feeding on field collected levels of Bt or non-Bt pollen for four days. However, first instar monarchs feeding on milkweed sprayed with insecticides or subject to exposure from insecticide drift were adversely affected. There was 90% to 100% mortality of monarchs feeding on milkweeds collected from within the field and 21% to 45% mortality from plants outside the field. This study suggests that Bt sweet corn may provide a safer habitat for monarchs than fields requiring insecticide applications (Stanley-Horne *et al.* 2001).

BPPD Conclusions:

The potential risk from other factors such as destruction of overwintering habitat, weather, predators, pesticides, physiological stress, and human activity (Taylor 1999) is a much greater and more widespread threat to monarch populations than the use of Bt corn. The risk Bt corn pollen poses to monarchs is probably relatively less than other significant risk factors especially insecticide use (Stanley-Horne *et al.* 2001). The potential reduction of insecticide use that may result from planting Bt corn will most likely benefit monarch populations as well as other beneficial insects.

DCI Data Element 20:

(20) Determine and report whether monarch populations travel linearly.

Classification: Acceptable

Summary of ABSTC's response:

Monarchs overwinter in Mexico and migrate to Gulf Coast states in late March. The first generation in the spring develops on southern milkweeds and emerge as adults in late April to early May. These adults migrate north and east across Midwestern states to Canada. As the monarchs migrate, they lay eggs and a second generation develops in the western and central Great Lakes in June. There is a midwest component of the population that migrates to the east over the Appalachians. A few monarchs migrate northeast of Florida along the coast. Two to three generations continue migrating north in the Midwest and east of the Appalachians before entering diapause. In the fall, diapausing monarchs migrate south back toward their overwintering site in Mexico. Monarchs complete a 360 degree rotation annually. The three to five generations progress through a continuous clockwise shift in the U.S. at a rate of one degree daily (Brower 1996).

BPPD Conclusions:

According to the summary of monarch migration submitted by the ABSTC as well as information found on the Monarch Watch website it can be concluded that monarch populations move linearly.

DCI Data Element 21:

(21) Confirm and report whether 50% of monarchs pass through the corn belt.

Classification: Acceptable

Summary of ABSTC's response:

Isotope analyses of monarchs that succumbed to "natural mortality" at 13 winter roost sites during February in Mexico was used to determine where they originated from. Wassenaar and Hobson (1998) showed that approximately 50% originated from an area extending from Nebraska and Kansas to Ohio, and 95% originated from the majority of the monarch breeding range. These results are based upon one year of sampling and may vary between years. In addition, these results should be considered with caution since only monarchs dying of natural mortality were considered. Although 50% of the monarchs may pass through the Corn Belt, it would take several years of evaluation to validate this conclusion (Taylor *et al.* 1999).

BPPD Conclusions:

The information submitted to the Agency thus far suggests that 50% of monarchs probably pass through the Corn Belt. Additional studies conducted over several years are needed to verify this conclusion. It would be useful to randomly sample the monarchs rather than only evaluating those that succumbed to natural mortality. However, this is not possible since live monarchs in the overwintering site should not be disturbed.

DCI Data Element 22:

(22) Develop and report a mathematical model to test the sensitivity of various environmental and biological risk factors, as well as to examine the risk to monarchs and other susceptible non-target insects at varying distances from Bt corn fields.

Classification: Acceptable

Summary of the ABSTC's response:

A monarch phenology-corn pollen release model was developed by Calvin *et al.* (2000). Environmental factors in this model include growing-degree-days which influence larval survival and pollen-shed. The sensitivity analysis of this model showed that the spring arrival of the northward migrating monarchs which are driven by environmental factors do not allow for predictions of early instar and corn pollination overlap.

A probabilistic model was developed by Pleasants *et al.* (1999) showing the potential risks to monarchs from Bt pollen flow off field. This model showed that any potential risk to non-target insects including monarchs would be restricted to inside the field or near the field edge.

In addition, the ABSTC references an USEPA physically based regulatory model (1995) that describes pollen flow in the environment as a function of pollen properties and environmental variables. According to the Calvin *et al.* (2000) model, the risk of monarch exposure to Bt pollen is negligible when pollen concentration near the field edge, emergence of various monarch larval instars, and timing of pollen shed are considered. Therefore, the ABSTC concluded that environmental factors such as light, moisture, and key period weather on spatial-temporal considerations are not necessary. The ABSTC also concluded that more highly sophisticated models are not necessary because there is no evidence of regional co-occurrence of a sensitive non-target insect.

Levels of corn pollen deposition on milkweed leaves are influenced by wind, wind direction, rainfall, time period pollen was sampled. In Pleasants *et al.* (2001) study, approximately 40% of pollen in the air was retained on leaves. Most of the pollen (86%) was removed from leaves after one 1.9 cm rainfall. Location of milkweed plants within corn fields or location of leaves on milkweeds also effect the number of deposited pollen grains. For example, 176.6 grains/cm^2 were found on milkweeds within corn rows and 118.9 grains/cm^2 were deposited on plants between rows. Pollen levels are higher 25 meters from the edge (147.5 grains/cm^2) than three meters from the edge (55.5 grains/cm^2). In addition, significantly more pollen was retained on middle and lower leaves than top leaves on the milkweed plant because of rainfall and , wind and/or plant movement. The increase pollen level on lower leaves may also be due to leaf orientation; upper leaves are situated more upright and are smaller than lower leaves (Pleasants *et al.* 2001).

<u>BPPD Conclusions</u>:

Models were based on growing degree units because it was the factor with the most influence on monarch development and corn phenology. Pleasants *et al.* (2001) found that levels of corn pollen deposition on milkweed leaves are influenced by wind, wind direction, rainfall and time period pollen was sampled. Corn pollen grains don't disperse far from it's source because they are large (~90 to 100 microns). In some instances, weather conditions such as thunderstorms and updrafts carry some pollen grains further than usual (Emberlin 1999). However, wind, rain, and other environmental factors will probably remove most of the pollen deposited on milkweed leaves (Pleasants *et al.* 2001).

It is difficult to report one specific quantity of corn pollen that will be deposited on milkweeds within corn fields and at varying distances from the field edge. Many factors influence pollen deposition and retention on milkweed leaves. Environmental factors such as rain and wind may increase the distance pollen will travel and may decrease the amount of pollen retained on leaves. Plant morphology such as leaf angle will also effect pollen deposition and retention. Upper leaves that are more upright and exposed to environmental factors retain less pollen than middle and lower leaves on the milkweed plant (Pleasants *et al.* 2001).

DCI Data Element 23:

(23) Define and report baseline monarch population levels and submit annual population level reports on a regional basis.

Classification: Acceptable

Summary of ABSTC's response:

The ABSTC has requested a waiver from this requirement because a baseline population level cannot be reasonably developed. It is difficult to develop a baseline population level using current methodology and because the number of monarchs throughout the U.S. fluctuates between regions and years. There are several factors such as catastrophic weather (e.g., drought or floods) that may adversely affect monarch population size. However, monarch populations may recover from catastrophes as is evidenced by the large number of monarchs counted in 1994, the year after floods in the midwest. On the other hand, warm summers result in increased population size in North America and decreased numbers during cold summers. The ABSTC lists other factors that may affect monarch population size, thus making it difficult to develop a baseline. These factors are: 1.overwintering site depletion, 2. number and fitness of monarchs that overwinter, 3. nectar availability to adults, 4. pathogens, parasites, parasitoids, and predators, 5. milkweed availability, 6. use of insecticides to control lepidopteran pests, and 7. accidents (e.g., collision with automobiles). Due to these factors, it is difficult to develop a baseline population size and if the number of monarchs fluctuates, there is no way to determine if Bt corn pollen was a contributing factor.

There have been several attempts made to determine monarch population levels. Swengel (1995) showed that from 1986-1994 there were significant changes in monarch counts including increases and decreases from five of eight year-pairs. Walton and Brower (1999) showed extreme variability in monarch counts in Cape May Point, NJ which is a major funnel point in September and October for monarchs migrating to Mexico. In Cape May, the 1999 counts were seven times greater than in 1998 and almost twice as high as any year since 1992 when the census began. In addition, the ABSTC points out that Monarch Watch has conducted annual surveys since 1993. Surveys from 1993-1999 are available online at www.monarchwatch.org.

BPPD Conclusions:

Based upon the facts discussed by the ABSTC, it can be assumed that it is not reasonably possible to develop a baseline monarch butterfly population size. However, it is possible to continue surveys such as the one conducted by the Monarch Watch to identify sudden, drastic decreases in the number of monarchs in North America and its overwintering sites in Mexico.

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